

Cytometric Bead Array

**Screening tool for the simultaneous detection of
pro-inflammatory cytokines and acute phase
proteins in porcine plasma**

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LPS



pro-inflammatory cytokines

TNF- α , IL-1 β , IL-6



fever

+

acute phase proteins

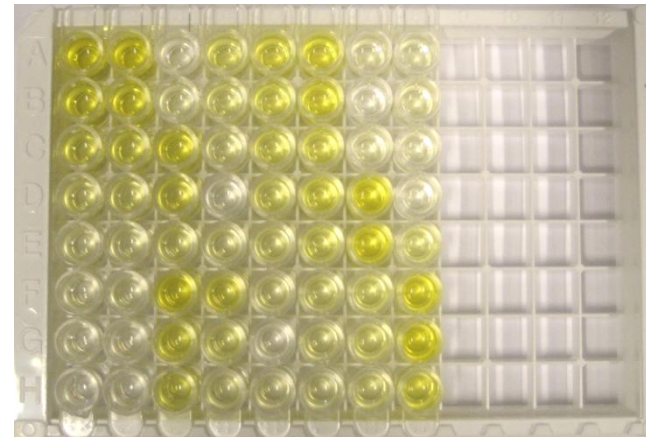
CRP, pig-MAP, Hp, SAA



ELISA

→ MOST FREQUENTLY USED

- 1 parameter
- time-consuming
- expensive

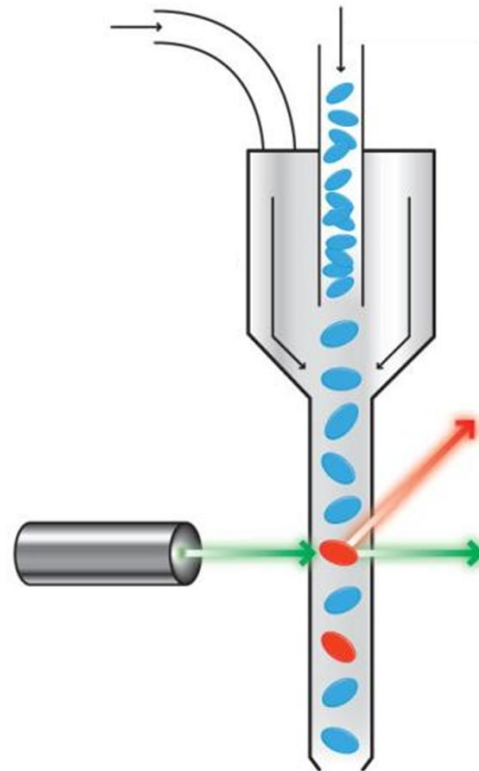


MULTI - PARAMETER ANALYSES ?

MULTIPLEX ASSAYS

→ BEAD-BASED FLOW CYTOMETRY

- **simultaneous detection**
- sample volume ↓
- analysis time ↓
- dynamic range ↑
- flexibility ↑

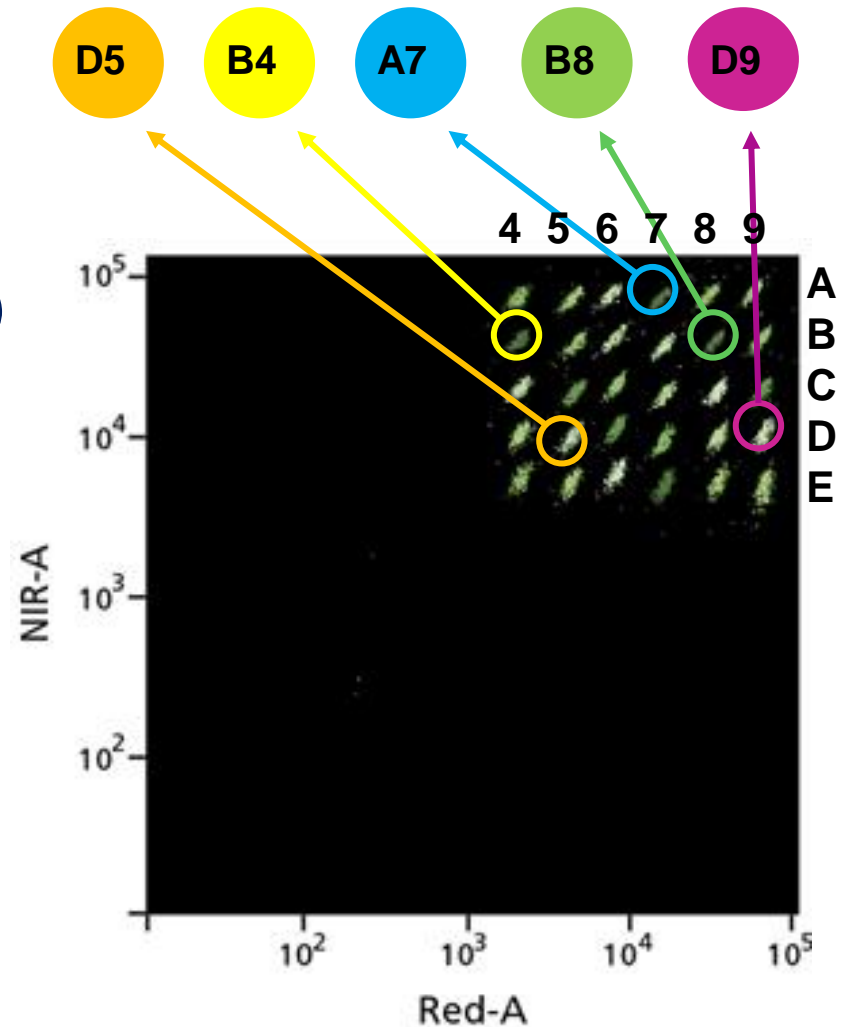


CYTOMETRIC BEAD ARRAY - CBA

→ BEADS

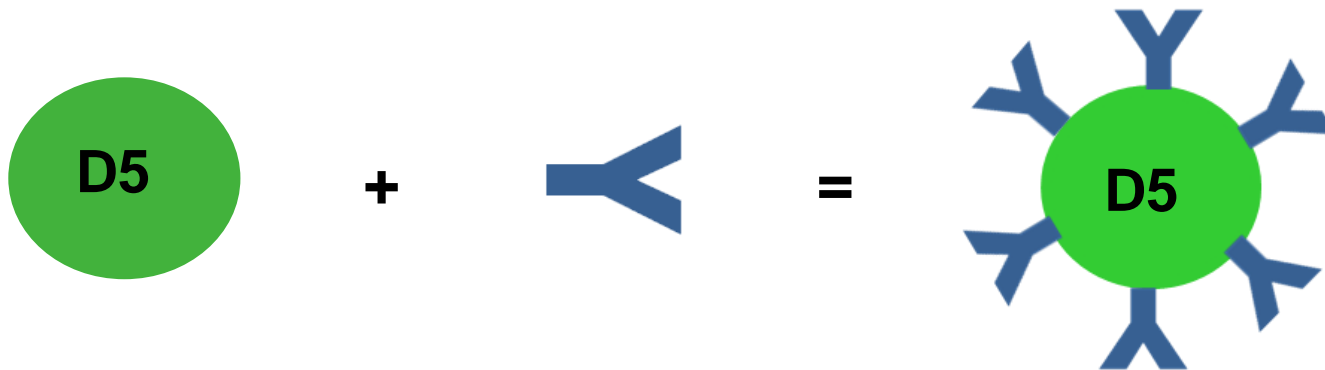
- same size and shape (7.5 μm)
- unique fluorescence intensity
- alphanumeric label

→ A4 to E9



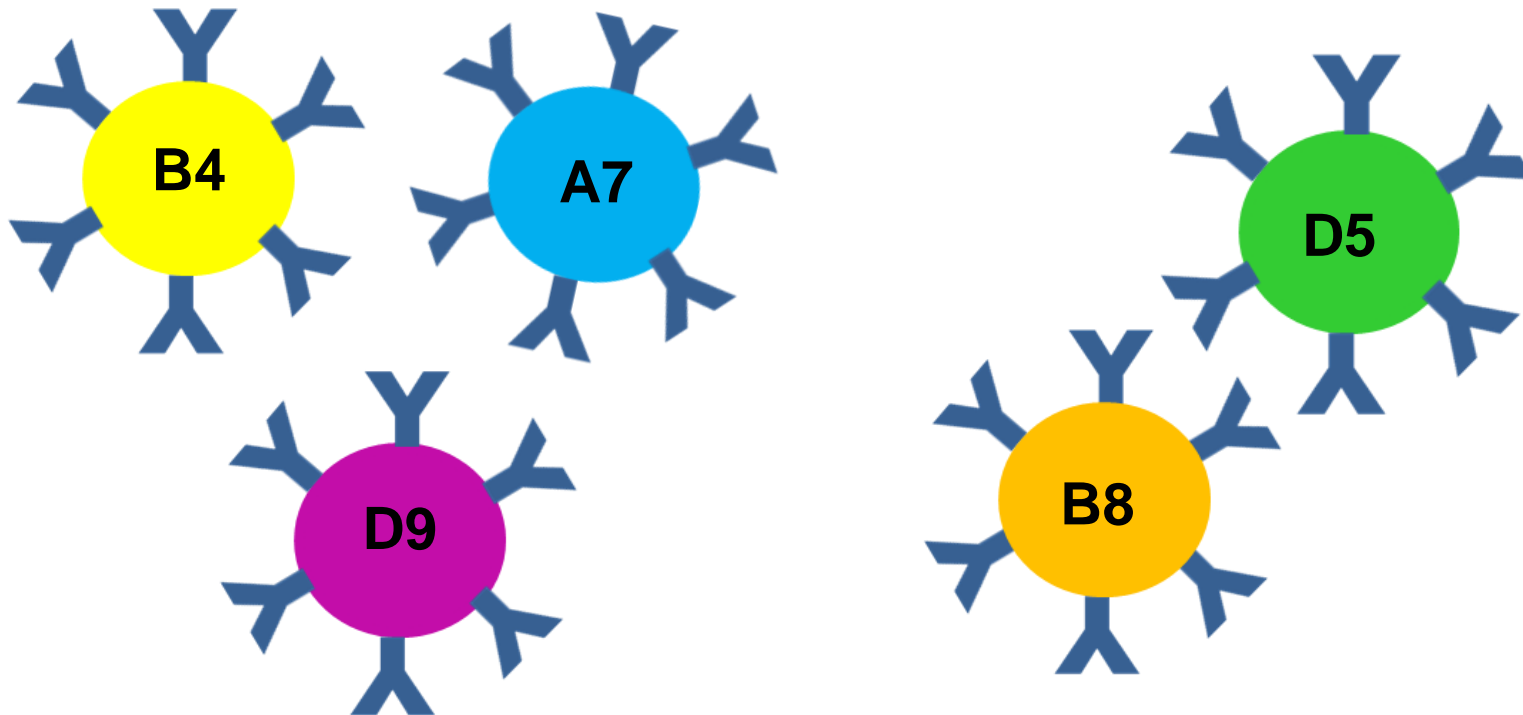
PROTOCOL

CAPTURE ANTIBODY (1)



PROTOCOL

CAPTURE ANTIBODY (2)



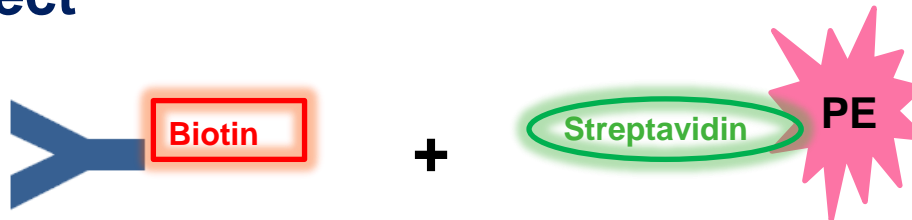
PROTOCOL

DETECTION ANTIBODY

a) Direct

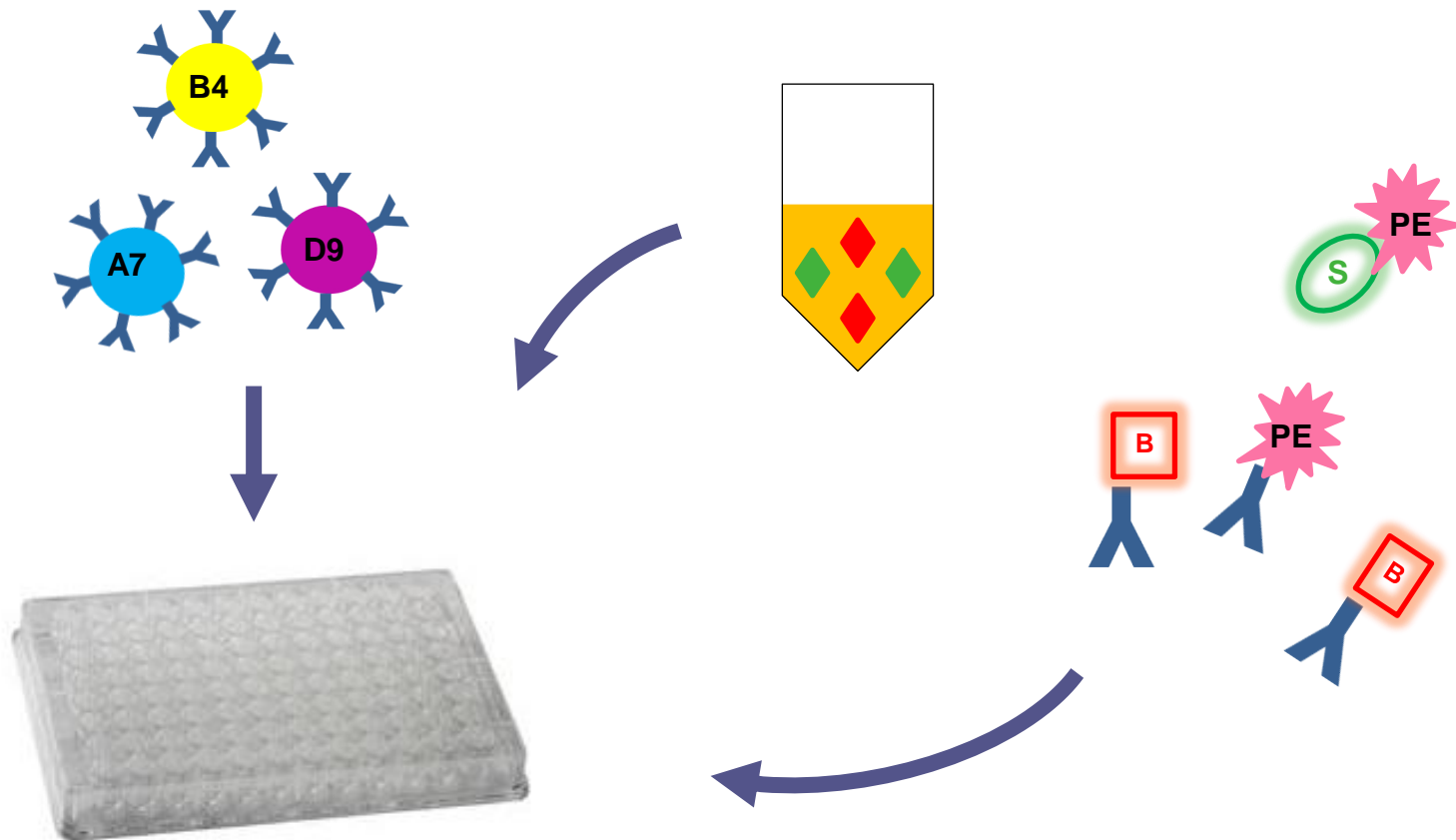


b) Indirect



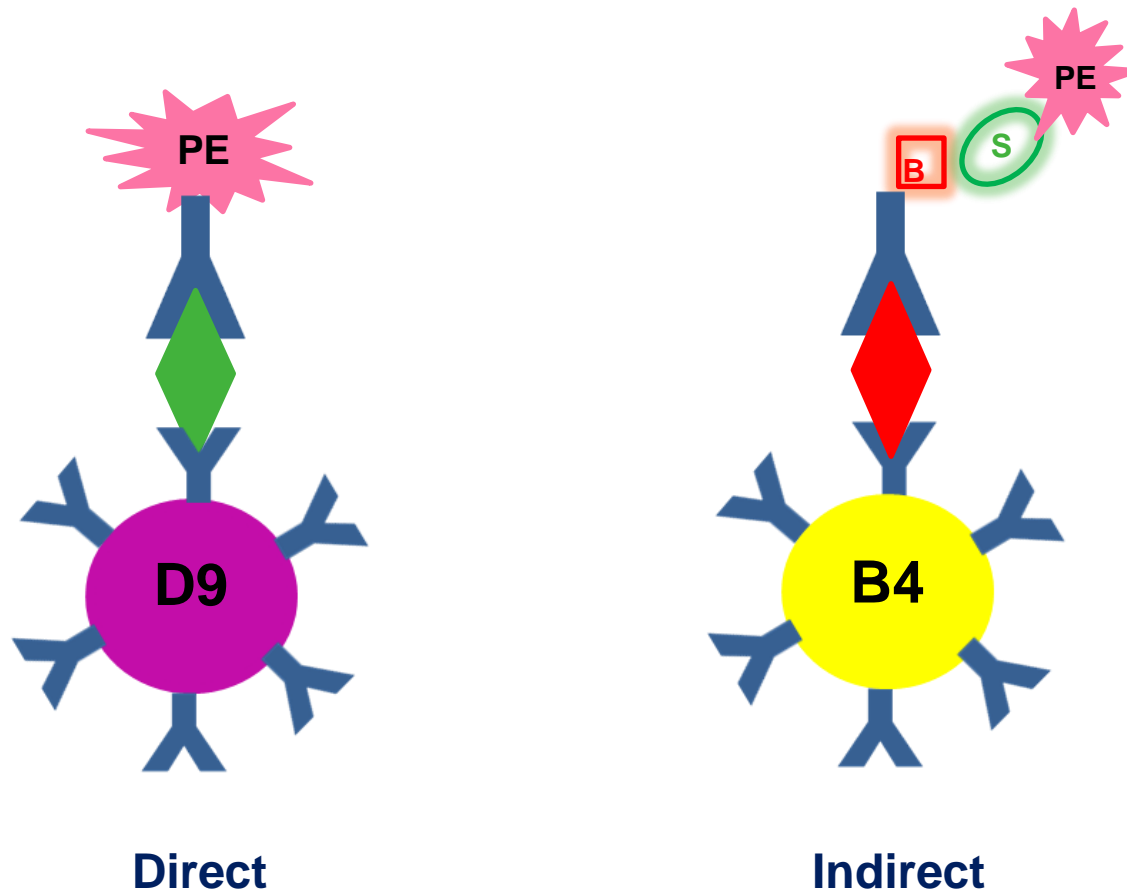
PROTOCOL

CBA MULTIPLEX ASSAY (1)



PROTOCOL

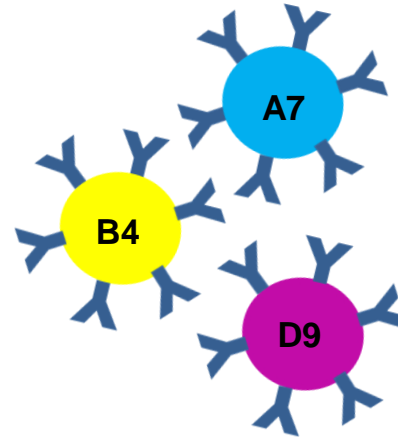
CBA MULTIPLEX ASSAY (2)



AIM

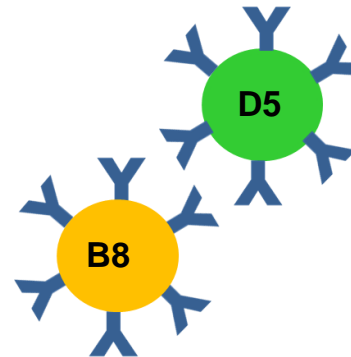
1) CBA 3-plex assay

- TNF- α
- IL-1 β
- IL-6



2) CBA 2-plex assay

- pig-MAP
- CRP



→ Comparison to ELISA: SIMILAR TREND?

ANIMAL EXPERIMENT

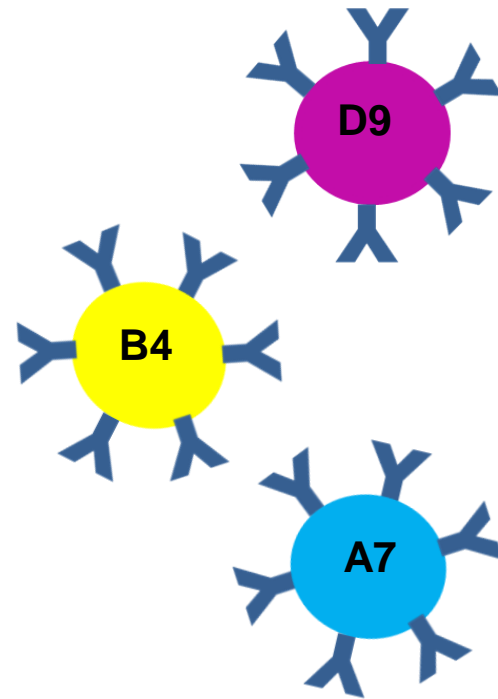
- Four 9-week-old, male pigs (25 kg)
- LPS (*E. coli* serotype O111:B4)
- 15 µg LPS/kg BW
- Blood samples



RESULTS

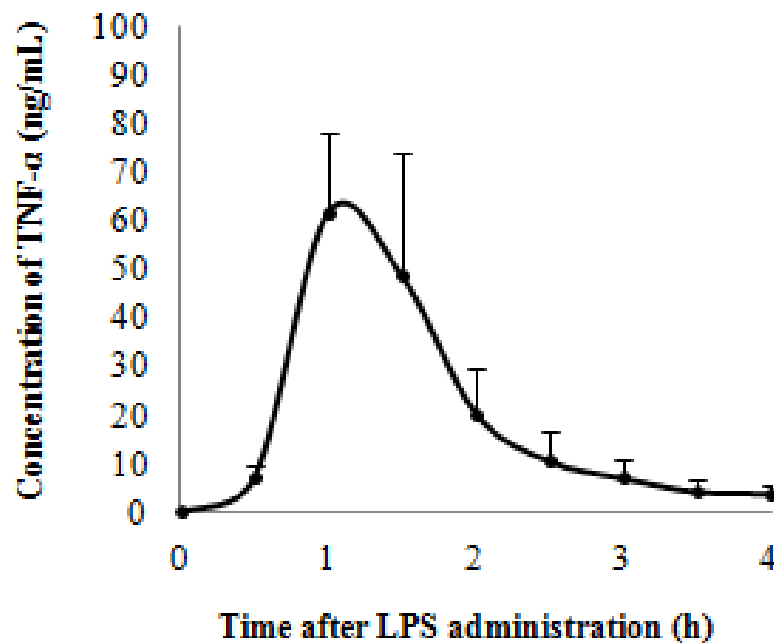
1) CBA 3-PLEX ASSAY

- **TNF- α**
- **IL-1 β**
- **IL-6**

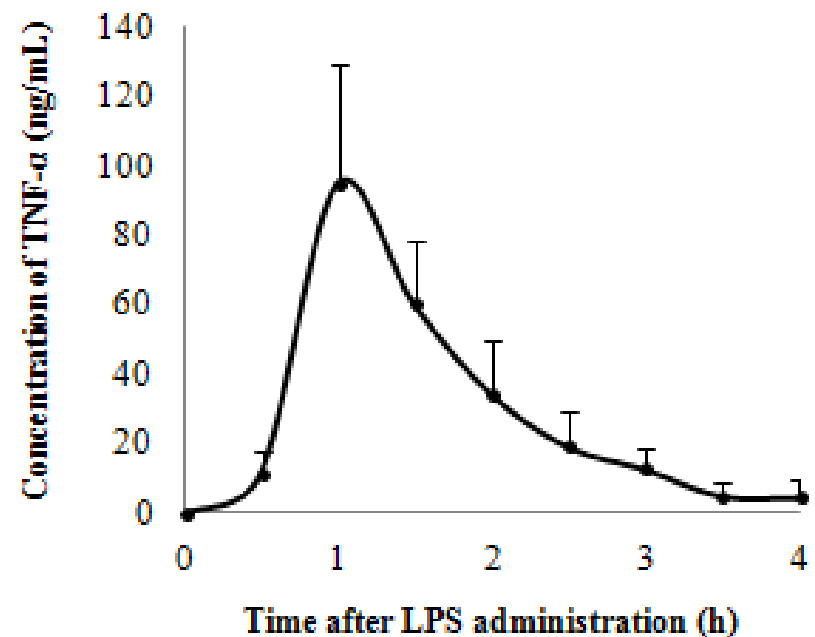


RESULTS

TNF- α



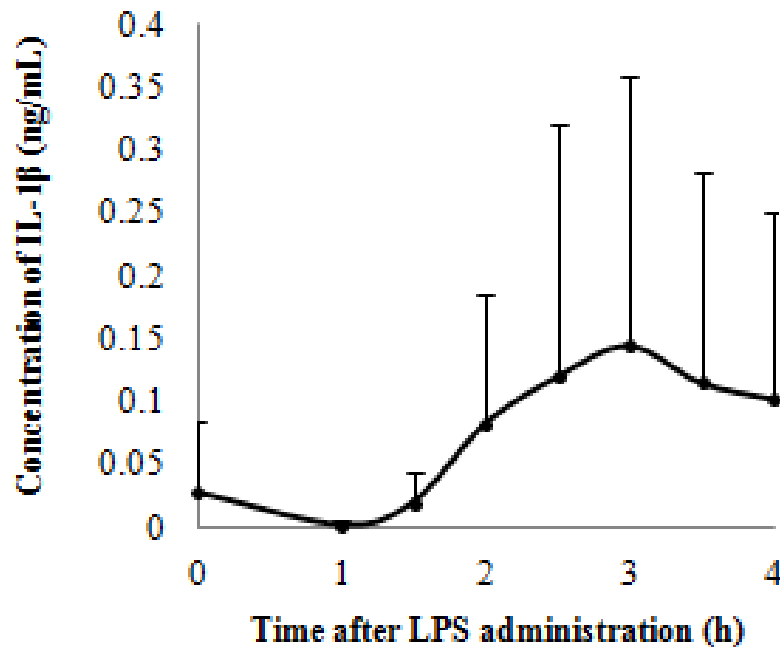
ELISA



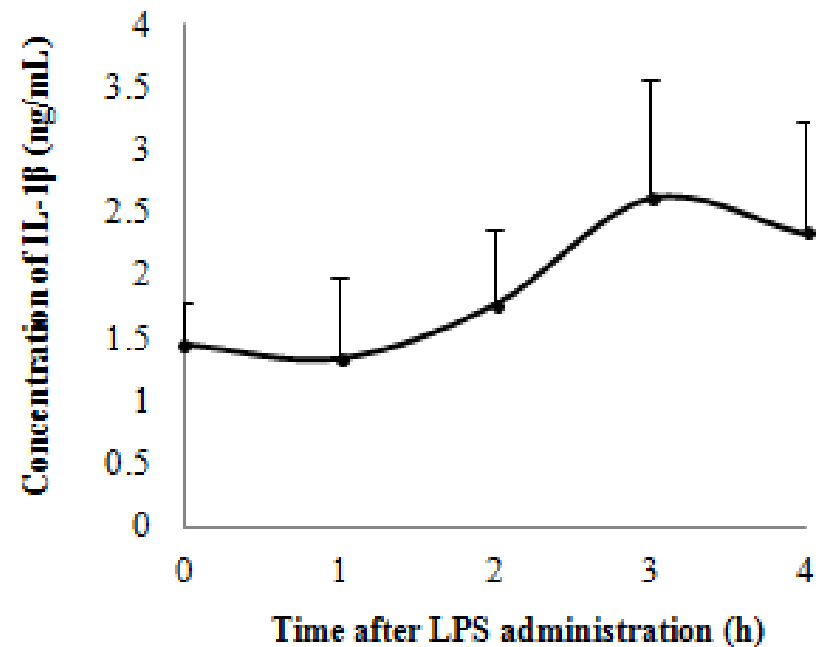
CBA 3-plex assay

RESULTS

IL-1 β



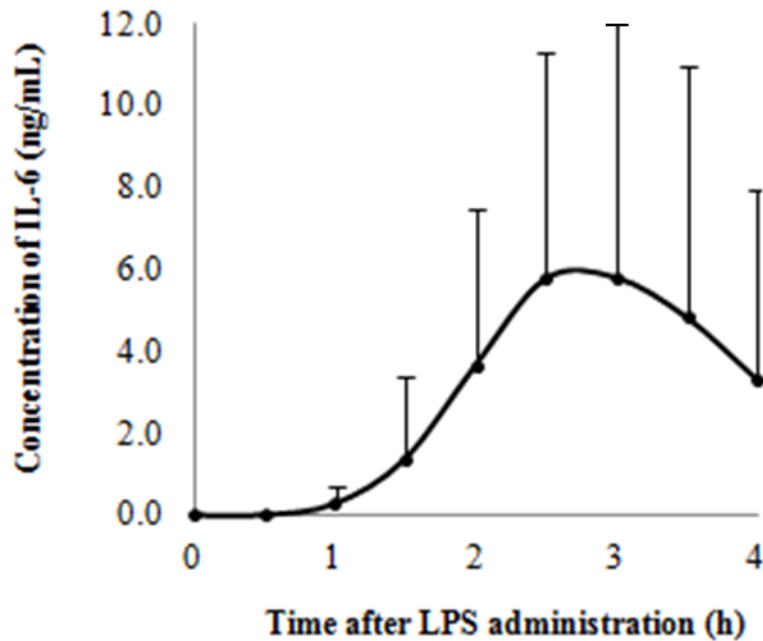
ELISA



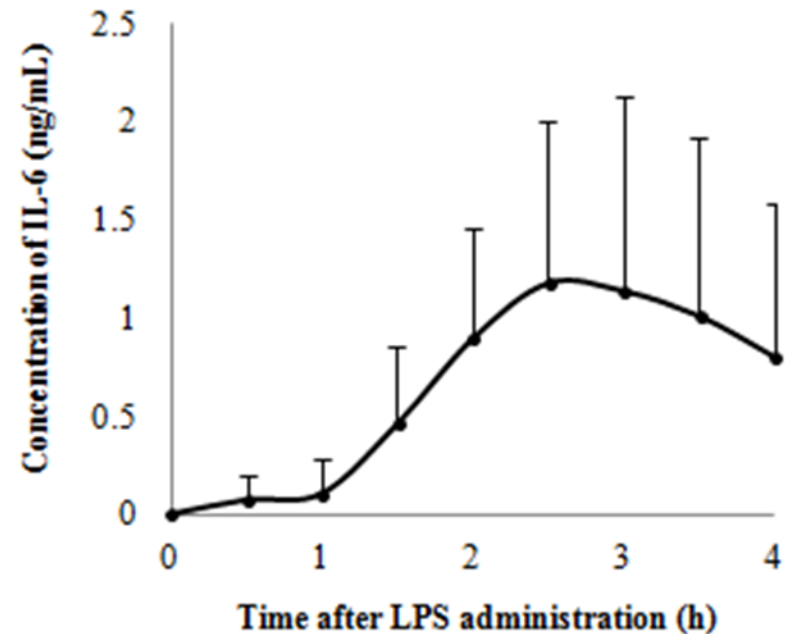
CBA 3-plex assay

RESULTS

IL-6



ELISA



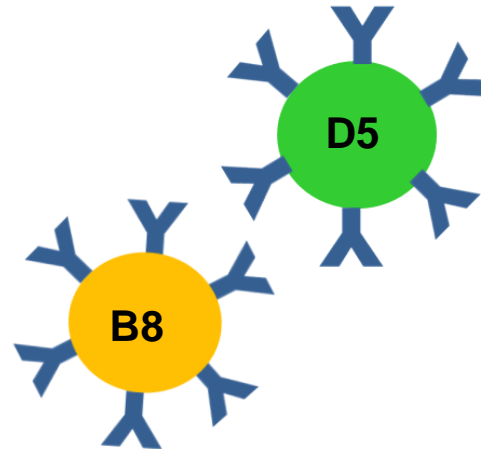
CBA 3-plex assay

RESULTS

2) CBA 2-PLEX ASSAY

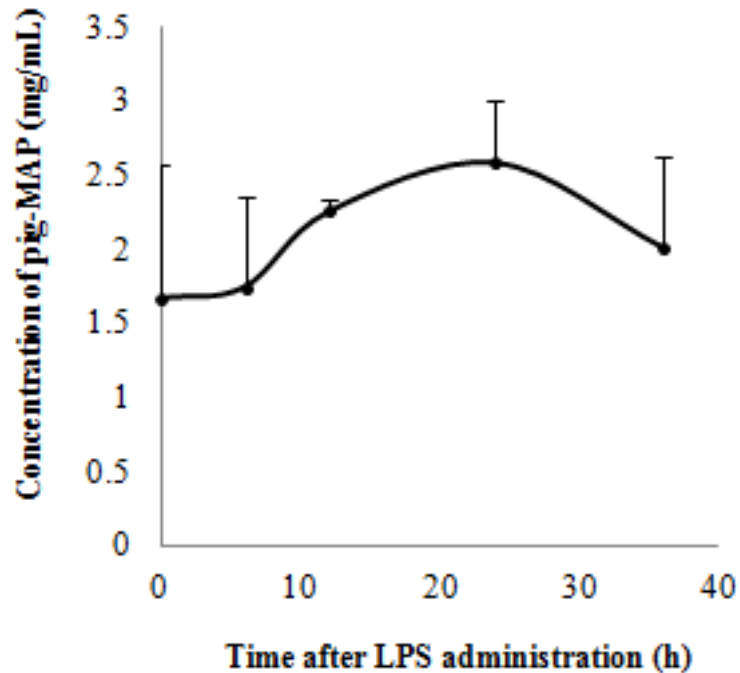
- pig-MAP

- CRP

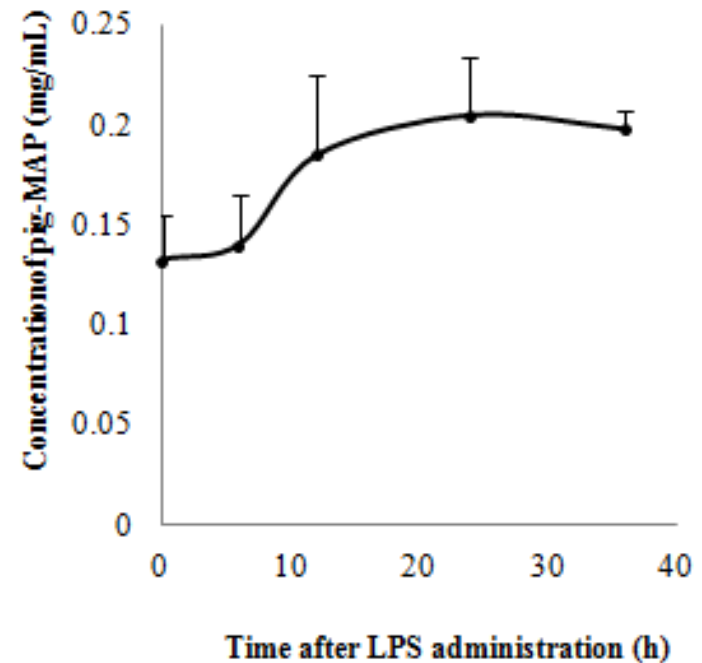


RESULTS

pig-MAP



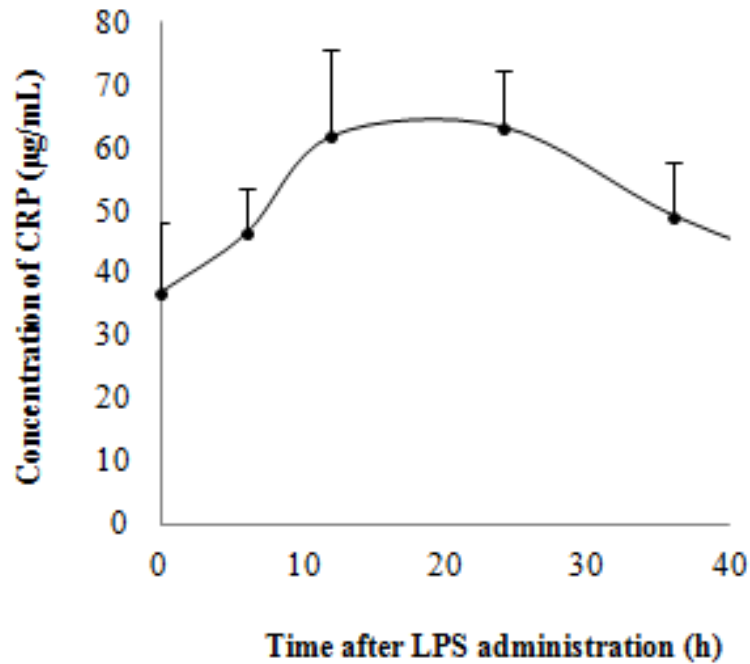
ELISA



CBA 2-plex assay

RESULTS

CRP



- **antibody pair:**

- succes on the **standard** level
- no succes on the **plasma** level

- **matrix interference?**

ELISA

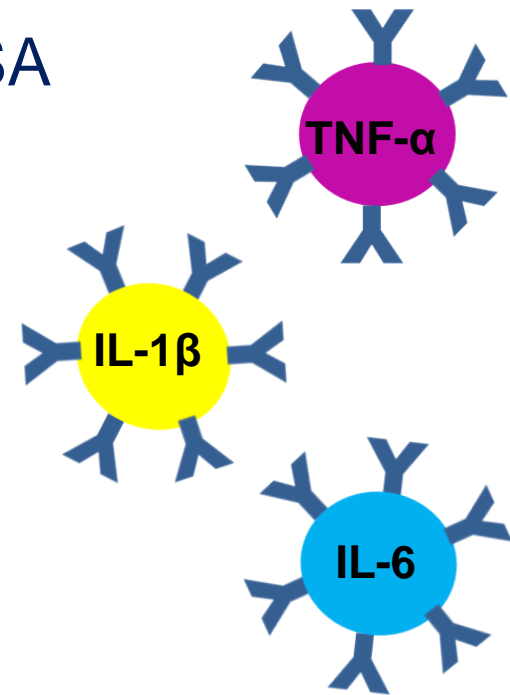
CBA 2-plex assay

CONCLUSIONS

1) CBA 3-plex assay

- cytokine profiles ~ ELISA
- fast
- reproducible
- cost-effective

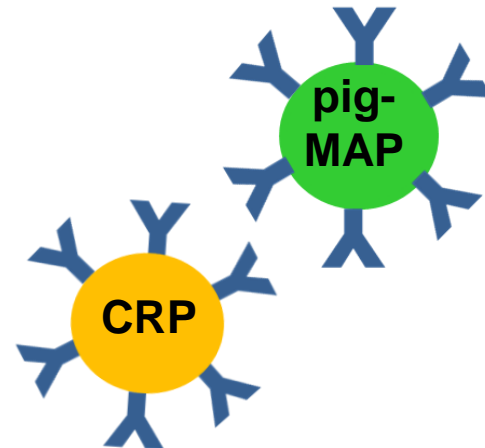
→ **AIMS FULFILLED**



CONCLUSIONS

2) CBA 2-plex assay

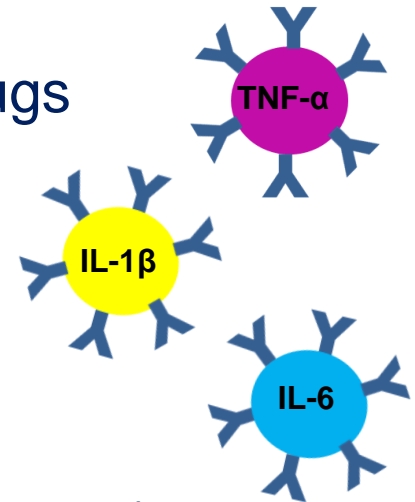
- profile pig-MAP ~ ELISA
- CRP not succesful



FUTURE RESEARCH

1) CBA 3-plex assay

- immunomodulatory properties of drugs
- effect on acute phase response?
 - fever ↓
 - pro-inflammatory cytokines ↓
 - acute phase proteins ↓

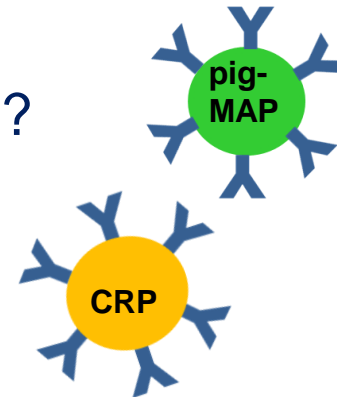


→ DEXAMETHASONE

FUTURE RESEARCH

2) CBA 2-plex assay

- alternative antibody pair for CRP?
- substitution by haptoglobin (Hp)?





Thank you for your attention!